

Norfloxacin Disposition After Sequentially Increasing Oral Doses

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Single doses of norfloxacin (200, 400, 800, 1,200, and 1,600 mg) or placebo were administered orally at weekly intervals to 14 healthy male volunteers in a double-blind study. Norfloxacin was measured in serum and urine by high-pressure liquid chromatography with UV detection. The concentrations of this drug in serum peaked 1 to 2 h after each dose; the mean peak values for increasing doses were 0.75, 1.58, 2.41, 3.15, and 3.87 $\mu\text{g/ml}$. Mean area under the serum concentration-time curves for the first 12 h after each dose were 3.56, 6.26, 11.4, 16.1, and 19.7 $\mu\text{g} \cdot \text{h/ml}$, respectively. The elimination half-life of norfloxacin was about 7 h and was similar for all doses. The concentrations of the drug in urine also peaked 1 to 2 h after dosage; mean peak values for increasing doses were 200, 478, 697, 992, and 1,045 $\mu\text{g/ml}$. Renal clearances approximated 285 ml/min. About 30% of each dose was excreted into urine as unmetabolized norfloxacin. Crystals of the drug were occasionally observed during microscopic examination of freshly voided urine collected after the 1,200- and 1,600-mg doses. Crystalluria was not encountered at lower doses.

Norfloxacin (MK-0366) is a new quinoline carboxylic acid which exhibits high antimicrobial activity in vitro against a wide variety of gram-negative and gram-positive bacteria, including gentamicin-resistant *Pseudomonas aeruginosa* and β -lactamase-positive *Neisseria gonorrhoeae* (2, 3, 5-7). In addition, this drug is severalfold more potent than nalidixic acid and pипemidic acid in the treatment of systemic and urinary tract infections in experimental animals (4). Initial clinical trials indicate that norfloxacin is effective in chemotherapy of urinary cystitis, pyelonephritis, and urethritis (Y. Nishimura, H. Kishi, O. Tsukada, T. Tominaga, and T. Nijima, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 76, 1980). The responsiveness of infections outside the urinary tract is not well characterized to date.

Although norfloxacin has been nontoxic in most laboratory animals, high doses of this drug (≥ 150 mg/kg) caused severe nephrotoxicity and crystalluria in dogs (Merck Sharp & Dohme Research Laboratories, personal communication). Rational selection of a dose regimen in humans will require detailed information on the oral dosage necessary for maintaining bactericidal drug concentrations in urine while minimizing the risk of in vivo crystalluria. To this end, we have measured norfloxacin in both urine

and serum over a wide range of acute oral doses and have assessed the potential risk of drug-induced crystalluria in humans.

MATERIALS AND METHODS

Volunteers. Fourteen male volunteers (aged 24 ± 2 years) participated in the study. All weighed within 10% of their ideal body weight (76 ± 8 kg) for their ages and heights and were judged to be in good health before the study on the basis of medical history, physical examination, and laboratory evaluation. They were instructed not to use other medications from 7 days before initiation of the study until completion of all treatment periods.

Study design. In a double-blind manner, volunteers were randomly assigned to receive each of seven oral treatments, including sequentially increasing doses of norfloxacin (200, 400, 800, 1,200, and 1,600 mg) and two randomly interspersed placebos. Volunteers received their medication (four capsules) with 250 ml of water in the morning after an overnight fast. They resumed a normal diet 3 h after drug administration. Blood from a forearm vein and urine were collected periodically for up to 48 h. During the first 6 h after each treatment, 10 ml of each freshly voided urine specimen was centrifuged at $2,500 \times g$ for 10 min, and the pellet was examined microscopically for the presence of crystals. A period of 6 days separated each treatment.

Analytical methods. The concentration of norfloxacin in urine and serum was measured according to published methods (1). Briefly, specimens were

adjusted to pH 7.5 with buffer and extracted with methylene chloride. The drug was concentrated by back-extracting into a small volume of 0.3 N NaOH and then was quantified by high-pressure liquid chromatography with UV detection at 273 nm. This method separates norfloxacin from its metabolites and provides reliable quantitation (i.e., less than 5% variation in replicates) down to 0.1 $\mu\text{g/ml}$ in serum and 1 $\mu\text{g/ml}$ in urine. Urine specimens were mixed well before assay to assure uniform sampling.

Area under the serum concentration-time curves (AUCs) were determined by the trapezoidal rule. Serum elimination half-lives were calculated from the slopes of the log concentration versus time plots from 6 to 12 h after dosage, where the slope = $-k_e$ and $t_{1/2} = 0.693/k_e$. Urinary elimination half-lives were calculated from the slopes of the log excretion rate (milligrams per hour) versus time plots from 8 to 48 h after dosage; the midpoint of each collection interval was used for computations. Renal clearances were calculated by dividing the amount of urinary norfloxacin (in micrograms) by the serum AUC (micrograms \cdot hour per milliliter).

To assess drug solubility in urine, norfloxacin was added in excess to urine samples that were previously adjusted to pH values between 4 and 10 with small quantities of 6 N HCl or 6 N NaOH. Urine was incubated at 37°C for 4 h with occasional interim adjustments in pH to correct for buffering effects of the drug. Drug-saturated urine samples were centrifuged at 37°C in an Eppendorf model 5412 high-speed centrifuge for 5 min, and the supernatant was assayed for

norfloxacin by high-pressure liquid chromatography. Some samples were cooled to 25°C after the 37°C incubation and were centrifuged at room temperature to simulate the temperature conditions that existed during microscopic examinations of clinical specimens.

Statistical comparisons between means were accomplished with two-way analysis of variance and Duncan's multiple range test. Variation in data is expressed as \pm standard deviation unless otherwise stated.

RESULTS

Mean serum concentrations of norfloxacin after various oral doses are shown in Fig. 1. Mean peak concentration (C_{max}) occurred between 1 and 2 h after drug administration, with a progressive delay in the peak as the dose increased (Table 1). The mean AUC (0 to 12 h) and C_{max} did not increase linearly with increasing dose. For example, the AUC/dose and C_{max} /dose ratios were significantly greater ($P < 0.01$) for the 200- and 400-mg doses than for the 1,600-mg dose. The serum elimination half-life of norfloxacin, based on 6- to 12-h data, was approximately 7 h and was similar for all doses. The elimination half-life as determined from renal excretion rates during the 8- to 48-h period was also about the same for all doses. Renal

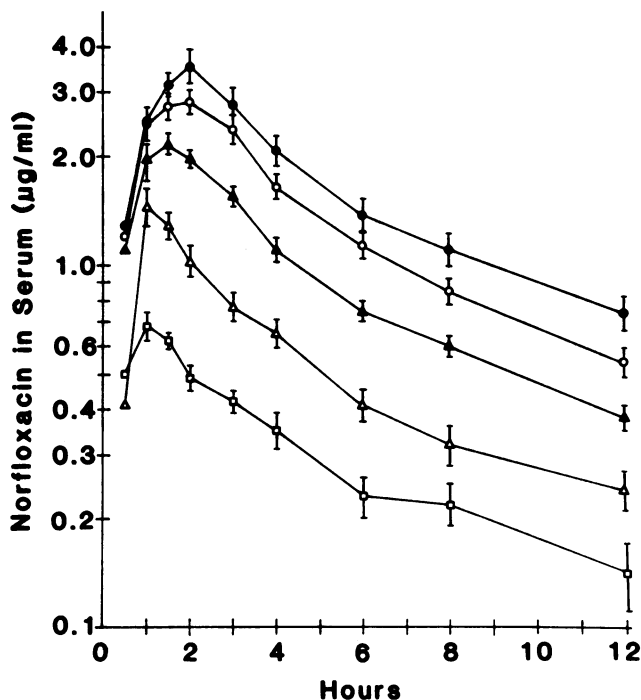


FIG. 1. Mean concentrations of norfloxacin in serum. Norfloxacin was administered orally at weekly intervals as single doses: 200 mg (□), 400 mg (△), 800 mg (▲), 1,200 mg (○), and 1,600 mg (●). Bars indicate the standard error of the mean.

TABLE 1. Pharmacokinetic parameters (mean \pm SD) for norfloxacin in 14 healthy men

Oral dose (mg)	Serum AUC ^a _{0-12 h} ($\mu\text{g} \cdot \text{h/ml}$) ^a	Serum C _{max} ($\mu\text{g/ml}$) ^b	Serum t _{max} (h) ^c	Serum t _{1/2} _{6-12 h} (h) ^d	Urinary t _{1/2} _{8-48 h} (h) ^e	Renal clearance 0-12 h (ml/min) ^f
200	3.56 \pm 1.13	0.75 \pm 0.20	1.1 \pm 0.4	7.3 \pm 2.9	7.1 \pm 1.2	272 \pm 96
400	6.26 \pm 2.05	1.58 \pm 0.60	1.3 \pm 0.4	7.4 \pm 2.5	8.0 \pm 2.4	292 \pm 76
800	11.4 \pm 2.12	2.41 \pm 0.43	1.5 \pm 0.4	6.2 \pm 1.8	7.9 \pm 1.6	273 \pm 37
1,200	16.1 \pm 4.00	3.15 \pm 0.76	1.8 \pm 0.8	5.7 \pm 1.2	7.1 \pm 1.1	288 \pm 56
1,600	19.7 \pm 6.07	3.87 \pm 1.27	1.9 \pm 0.6	6.8 \pm 1.4	6.7 \pm 1.2	298 \pm 40

^a AUC^a_{0-12 h}, Area under the curve from 0 to 12 h after drug administration.

^b C_{max}, Peak concentration.

^c t_{max}, Time of peak concentration.

^d t_{1/2}_{6-12 h}, Half-life with 6- to 12-h data.

^e t_{1/2}_{8-48 h}, Half-life with 8- to 48-h data.

^f Renal clearance = drug excreted/serum AUC (0- to 12-h data).

clearances were large (about 285 ml/min), and urinary drug concentrations were consequently 100- to 300-fold greater than concurrent concentrations in serum (Table 2). Approximately 30% of each dose was excreted into urine as unmetabolized norfloxacin in the first 48 h after drug administration (Fig. 2).

The urinary solubility of norfloxacin was found to be dependent on both pH and temperature (Fig. 3). The drug was least soluble at pH 7.5, exhibiting a maximal solubility of about 450 $\mu\text{g/ml}$ at 25°C and 1,200 $\mu\text{g/ml}$ at 37°C. At 37°C, the drug was freely soluble (concentration >40 mg/ml) at any pH value less than or equal to 5.5. Drug crystals were observed in only 5 of over 300 clinical specimens. All five specimens had been collected after 1,200- or 1,600-mg doses and exhibited pH values between 7.0 and 7.8 and drug concentrations between 1,200 and 2,300 $\mu\text{g/ml}$. Drug crystals in urine were spherical with ragged edges and orange and green highlights. This was in marked contrast to crystals precipitated from water, which were colorless needles.

Routine laboratory tests for proteinuria, blood urea nitrogen, and serum creatinine indicated that acute doses of norfloxacin were not nephrotoxic. For example, the values for blood urea nitrogen and serum creatinine were 13.7 \pm 3.9 and 1.10 \pm 0.12 mg per 100 ml, respectively, before the first dose and were 14.8 \pm 3.6 and 1.06 \pm 0.09 mg per 100 ml 3 days after the last dose.

DISCUSSION

This study has defined several important features of norfloxacin disposition after oral administration. Maximal concentrations in serum and urine were rapidly achieved, typically within 1 to 2 h. Assuming that norfloxacin is 14% bound to serum proteins (Merck Sharp & Dohme Re-

search Laboratories, personal communication), the free renal clearance for this antibiotic was about 325 ml/min, a remarkable 2.5 times the normal glomerular filtration rate. Presumably, norfloxacin is actively secreted as an organic acid. However, this antibiotic is both an acid and a base and could also be secreted by other mechanisms. The amphoteric nature of the drug is reflected in its urine solubility curve (Fig. 3), which shows a trough in solubility at pH 7.5 and greatly enhanced solubility under both more acidic and more basic conditions.

With rising doses, the serum C_{max}, serum AUC from 0 to 12 h, and the 0- to 12-h urinary recovery of unmetabolized drug all became progressively lower relative to the amount of drug administered. This suggested that the fraction of the dose absorbed over the first 12-h period was lower at the higher doses. However, by 48 h, the mean percent recovery in urine was not substantially different for the various doses (range, 27.4 to 31.0%), indicating that absorption may have been delayed at the higher doses. These dose-related changes in norfloxacin bio-availability may be of no clinical importance, as they occurred at excessively high doses. Although the apparent serum half-life of norfloxacin in our study was found to be approximately 7 h, accurate calculation of an elimination half-life in serum is not possible if drug absorption is substantially delayed. However, approximately the same half-life can be derived from urinary excretion rates observed after drug absorption processes are complete.

In our study, drug-related crystals were observed only at the highest (1,200 and 1,600 mg) doses of norfloxacin and only when urine pH values exceeded 7.0. Since norfloxacin appears to be clinically effective at a total daily dose of less than 600 mg (20th ICAAC, abstr. no. 76), crystalluria will probably not pose a significant problem during chemotherapy with this agent at

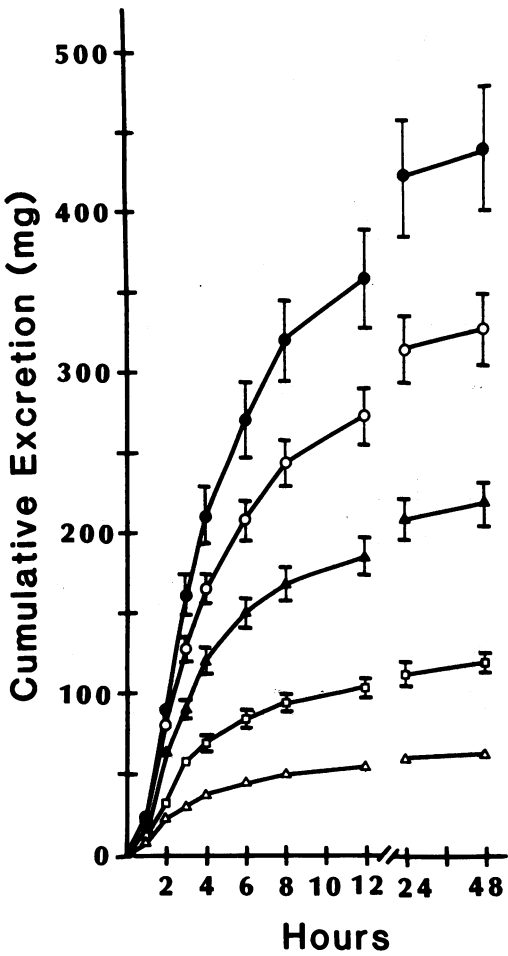


FIG. 2. Cumulative urinary excretion of unmetabolized norfloxacin after various single oral doses: 200 mg (Δ), 400 mg (\square), 800 mg (\blacktriangle), 1,200 mg (\circ), and 1,600 mg (\bullet). Points and bars indicate means \pm standard errors of the mean.

these lower doses. The urinary concentration data indicate that the 1,200- and 1,600-mg doses should not be required in the treatment of urinary tract infections, as the minimal inhibitory concentration of norfloxacin for most pathogens is $<4 \mu\text{g/ml}$ (2-7); this concentration was exceeded in all urine specimens collected between 0 and 12 h after even the 200-mg dose.

This study in normal volunteers has shown that norfloxacin rapidly reaches therapeutic concentrations in urine after oral administration and that drug-induced crystalluria is unlikely to occur at usual therapeutic doses. Future studies on norfloxacin disposition should be undertaken to assess the extent of drug cumulation and the potential risk of crystalluria after chronic administration.

TABLE 2. Mean concentrations of norfloxacin in urine of 14 healthy men at various times after dosage

Dose (mg)	Mean (SEM) norfloxacin concn ($\mu\text{g/ml}$) at (h):									
	0-1	1-2	2-3	3-4	4-6	6-8	8-12	12-24	24-48	
200	133 (37.2)	124 (17.9)	88.5 (24.8)	75.6 (18.3)	48.1 (6.55)	44.0 (7.27)	19.7 (3.71)	10.3 (0.99)	2.35 (0.48)	
400	168 (42.0)	417 (157)	299 (98.6)	211 (44.9)	141 (26.3)	100 (21.7)	46.9 (9.97)	22.5 (3.34)	5.93 (0.82)	
800	280 (68.7)	582 (126)	365 (74.9)	358 (55.1)	243 (36.3)	184 (26.1)	84.4 (14.2)	45.9 (5.61)	12.1 (2.75)	
1,200	359 (119)	897 (201)	657 (110)	550 (93.6)	424 (80.0)	272 (43.3)	143 (26.4)	73.5 (14.6)	15.7 (1.85)	
1,600	250 (58.0)	881 (193)	625 (147)	421 (89.6)	518 (131.3)	405 (108)	188 (34.2)	101 (16.0)	19.1 (2.34)	

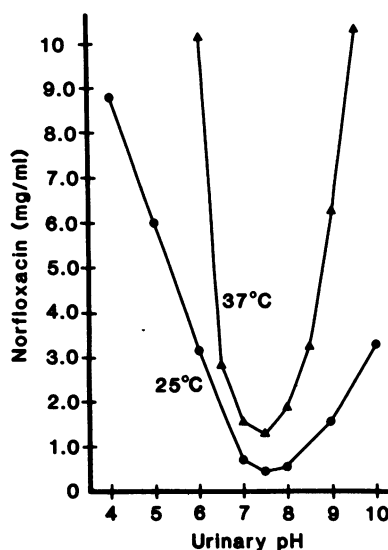


FIG. 3. Effect of pH and temperature on urinary solubility of norfloxacin.

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